Nasal temperature drop in response to a playback of conspecific fights in chimpanzees: A thermo-imaging study

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Abstract

Emotion is one of the central topics in animal studies and is likely to attract attention substantially in the coming years. Recent studies have developed a thermo-imaging technique to measure the facial skin temperature in the studies of emotion in humans and macaques. Here we established the procedures and techniques needed to apply the same technique to great apes. We conducted two experiments respectively in the two established re- search facilities in Germany and Japan. Total twelve chimpanzees were tested in three conditions in which they were presented respectively with the playback sounds (Exp. 1) or the videos (Exp. 2) of fighting conspecifics, control sounds/videos (allospecific display call: Exp. 1; resting conspecifics: Exp. 2), and no sound/image. Behavioral, hormonal (salivary cortisol) and heart-rate responses were simultaneously recorded. The nasal temperature of chimpanzees linearly dropped up to 1.5 °C in 2 min, and recovered to the baseline in 2 min, in the experimental but not control conditions. We found the related changes in excitement behavior and heart-rate variability, but not in salivary cortisol, indicating that overall responses were involved with the activities of sympathetic nervous system but not with the measureable activities of the hypothalamus–pituitary–adrenal (HPA) axis. The influence of general activity (walking, eating) was not negligible but controllable in experiments. We propose several techniques to control those confounding factors. Overall, thermo-imaging is a promising technique that should be added to the traditional physiological and behavioral measures in primatology and comparative psychology.

Introduction

Many researchers agree that emotion is, and will be, one of the central interests in the study of animal behavior [1,2]. Yet, one barrier to the emotion studies in nonhuman animals is the scarcity of reliable, handy, and noninvasive tools to measure their physiological states [3]. In non- human primates, most of the previous studies used the directly- observable responses to infer the internal state of animals, such as facial expressions, bodily postures, vocalization, self-directed scratching, piloerection, and urination/defecation [as described in "ethograms" e.g. [4,5]]. Endocrine responses, especially cortisol secretion through hypothalamus–pituitary–adrenal (HPA) axis, are also commonly used to measure stress levels in primates. Cortisol or cortisol metabolites can be measured in urine, feces, and saliva [6-13]. These methods are noninvasive, and the sampling does not interfere with natural behaviors of animals; thus they are widely adopted in both laboratory and field studies. Currently lacking is a handy tool for measuring the activities of autonomic nervous system in nonhuman primates.

In humans, the autonomic-nervous-system (ANS) measures are commonly used to study emotion because these measures are well established and easily applicable, and are distinguishably related to a unique feeling, such as anger, fear, happiness, sadness [14,15]. ANS is in- volved with the changes in heart-rate or heart-rate variability, pulse rate, blood pressure, pupil size, galvanic skin-conductance, respiratory rate, and skin temperature. ANS has two branches: the sympathetic nervous system (SNS) and the parasympathetic nervous system (PSNS), and these two systems typically act as antagonists. SNS is dominant when a body is preparing for impending risks/dangers while PSNS is dominant when a body is at rest [16,17]. In nonhuman primates, several attempts were made to measure SNS activities to estimate their internal psychological states. Boysen and Berntson [18] used electrocardiogram to measure heart-rate of a young chimpanzee and found that they accelerated their heart-rate in response to the pictures of an aggressive con- specific. Berntson et al. [19] used the same method and found that chimpanzees decelerated their heart-rate in response to the pictures of familiar caretakers and the sounds of conspecific screams, presumably due to their increased attention to the stimuli. Aureli et al. [20] used the same technique (but wirelessly transmitted the signals to the recorder) to measure heart-rate of freely-moving macaques and found that the monkeys increased their heart-rate in response to the approaches by dominants. The monkeys decelerated their heart-rate faster during the receipt of grooming than matched control periods. Parr [21] used temperature transducer attached to a finger to measure the finger skin temperature of chimpanzees, and found that the finger temperatures dropped in response to a potentially threatening stimulus (a needle and a conspecific being injected with a needle). Although these studies were insightful, an unavoidable limit of their methods is that the subjects need to accept electrodes on their bodies. This typically results in a small number of testable (tolerant) subjects and a restricted movement of subject due to the attached electrodes (and cables).

Recent advance in thermo-imaging techniques offer a contact-free method of autonomic-nervous-system measures in humans and nonhuman primates and thus have potentials to handle with the above-mentioned issue. In humans, depending on the elicited emotion and the task nature, skin temperature changes in specific regions of face/body in a specific way [for a review, see Ioannou et al. [22]]. In general, negative emotion such as fear and stress decreases the nasal skin temperature primarily due to the reduced blood flow resulting from vasoconstriction of subcutaneous vessels in

nasal skins [22]. Pain [23], startle [24], social pressure [e.g. public speech [25], accidental breaking of somebody's toy [26]], play [26,27], empathy [e.g. seeing the person in a stress [28]], and the stress associated with task execution [e.g. driving [29], numeric task [30]] decreased the nasal (and perinasal) temperature. Sexual arousal, induced by intimate touch [31], interpersonal proximity and eye contact [32], and viewing erotic movies [23], caused the opposite effect; the increase in nasal (and perioral) temperature due to the increased blood flow. In monkeys, presentation of potentially fearful or stressful stimuli, such as a threatening experimenter, videos of screaming and threatening monkeys, caused the de- crease in nasal temperature [3,33]. In the same studies, behavioral responses and simultaneously-recorded galvanic responses of monkeys were correlated with the decrease in nasal temperature. Although the monkeys were restrained in chairs in these studies, a recent study [34] showed a possibility in using thermo-imaging with freely-moving monkeys. In great apes, there is no study that used thermo-imaging to examine their internal psychological states.

Therefore, in this study, we aimed to establish the procedures optimized to measure the facial temperature (with a particular focus on nasal areas) of great apes using thermo-imaging and then to estimate their psychological responses to social stimuli. We focused on chimpanzees, one of the most common species in the studies of cognition and emotion. We aimed to replicate and extend the results from Kuraoka and Nakamura [3] and Nakayama et al. [33], in which the tested monkeys showed the decrease in nasal temperature to potentially threatening stimuli. Specifically, (1) we aimed to establish the optimized procedures in two of the research facilities which noninvasively study apes, (2) to measure the change in nasal temperature when chimpanzees were hearing/watching various auditory and visual stimuli which differs in the degree of potential psychological impacts (e.g. conspecific fighting, conspecific resting, vs. no event) (3) to find the response and recovery speed of change in nasal temperature, (4) to find behavioral, hormonal, and heart-rate (HR)/heart-rate variability (HRV) correlates, and (5) to find the potential confounding factors that may have influenced the nasal temperature independently of the stimuli. We expected that, according to the results by Kuraoka and Nakamura [3], chimpanzees would show the decrease in nasal temperature in response to potentially threatening stimuli. The response and recovery speed should be moderate (e.g. detectable changes within 30 s after the stim- ulus onset/offset). The degree of temperature drop should depend on the potential psychological impacts of stimuli. During the presentation of potentially threatening stimuli, chimpanzees should exhibit the in- crease in excitement behavior, and the decrease in HRV as well as the decrease in nasal temperature. These changes (related to sympathetic nervous activation) may even lead to an activation of the HPA axis and an increase in salivary cortisol levels. The potential confounding fac- tors should be any physical activities that trigger the sympathetic nervous activation [e.g. walking [35], food consumption [34,36]] as well as the environmental temperature when/where the test would be conducted.

Experiment 1

Using playback stimuli, Experiment 1 examined the change in nasal temperature with nine chimpanzees in Germany. Chimpanzees were tested in three conditions, in which they heard 1) fighting vocalizations by groupmate individuals (screams and barks), 2) display calls by an allospecific individual, or 3) no sound in the testing room. As six of the tested chimpanzees were already trained to chew the oral swabs and re- turn them to the experimenter on request, we

additionally collected the saliva samples from them for salivary cortisol analysis. It was expected that chimpanzees show the decrease in nasal temperature, the increase in excitement behavior, and the increase in salivary cortisol level, particularly when hearing a playback of fighting. These changes should be moderate or absent when hearing a playback of allospecific individual or no sound. The thermal and salivary cortisol measures were conduct- ed before the stimulus onset (baseline) and after the stimulus offset (post-measurement and recovery periods), but not during the stimulus presentation to allow chimpanzees to behave freely in response to the stimuli. We considered the chimpanzees' locomotive activities during the stimulus presentation as a potential confounding factor which could affect the nasal temperature independently of conditions.

Method

Participants

Nine chimpanzees (four females, five males; age range 9–39 years) participated in this study (Table S1). All chimpanzees were naïve to the procedures of thermal analysis. Six of the nine chimpanzees (two females, four males; Table S1) were previously trained for the purpose of saliva collection [6]. All chimpanzees are kept in Wolfgang Köhler Primate Research Center (WKPRC), Germany. On each day of testing, each chimpanzee was called into a separate testing room, where various cognitive tests were conducted on a routine basis.

Ethics statement

Chimpanzees have access to indoor and outdoor enclosures. These enclosures are well structured for chimpanzees to rest, exercise, and socialize with the groupmates. The chimpanzees received fresh fruits, vegetables, nuts and leaves distributed in three main meals and occasional enrichment programs. Water was available ad libitum throughout the day. The chimpanzees voluntarily participated in this study. Animal husbandry and research complied with the international standards and the local guidelines which are strictly adhered to the national laws of Germany. See Supplemental material for these details.

Stimuli

We prepared three 130-s auditory files as stimuli respectively for three conditions. In the "chimp fighting" condition, the file contained the vocalizations of groupmate chimpanzees recorded during a severe "grab and bite" fight among them. These consisted of screams and "waa"-barks of multiple individuals (Movie 1). In the "orangutan long- call" condition, the file contained long-call of a male orangutan. The participant chimpanzees customarily heard this vocalization from their living areas and usually did not overtly respond to it. In the "no sound" condition, the file recorded no sound (a blank file with the same duration). A hidden speaker (Logitech Z120BW) played the auditory stimuli as if those sounds came from the outside of room in real-life. The playback intensity ranged about 60–70 dB which was measured about in the same position of participant chimpanzees. See Movie 1 and Fig. S1 for examples of stimuli.

Procedure

Thermal measurement. A high-spec infrared thermo-camera (T650sc, FLIR, Stockholm, Sweden; a temperature sensitivity of 0.02 K; a resolution of 640 × 480 pixels) recorded the facial areas of chimpanzees with a 24.6-mm lens at a sampling rate of 0.13 s (7.5 fps). A laptop (Dell ES440) and the FLIR ResearchIR Software controlled the camera and stored all thermal data in an external hard drive. According to the system recommendation, we booted the camera at least 15 min before the start of recording.

The thermo-camera aimed at the chimpanzee's face through a metal mesh with 6 × 6-cm grids (partially cut) which separated the chimpanzees from the experimenters/apparatuses. The camera was placed on a tripod and at about 1 m from the mesh. To keep a constant distance be- tween the camera and the chimpanzees' face at the time of measurement, an experimenter gave a small piece of fruit (about 1− 2 cm^3) to the chimpanzee periodically through the mesh (every 3−5 s; but not during the stimulus playback; note that the thermal data cannot be collected through an acrylic panel or a glass due to the reflection of thermal images). Preliminary sessions confirmed that consumption of those foods did not affect their nasal temperature. See Movie 1 for an ex- ample of these procedures.

Each testing session lasted for about 15−20 min for the thermal measurements (and for an additional 20 min for the saliva collection). Only one session (the presentation of a stimulus) was conducted on each day for each participant. All sessions were conducted between 10−12 am, in May−June 2014. The testing room was kept in a constant temperature and humidity throughout a whole session, with no direct sunlight or ventilation. The temperature and humidity varied 20−25°C and 50− 80%, respectively, across the testing days. Before testing, the chimpanzees stayed in an indoor compound where the temperature and humidity was similar to the testing room's. In most of the testing days, when the chimpanzees were called into the testing room, their nasal temperatures were lower than the other areas of face, probably due to their excitement and physical (locomotive) activities accompanying with the room entrance. Therefore, we set a 10−15 min resting period before each daily session to allow their nasal temperature to be recovered. During this resting period, the experimenter gave fruit pieces periodically to chimpanzees through the mesh until the chimpanzees show no behavioral sign of excitement or anxiety.

The daily session was divided into three phases, baseline (30 s), stimulus-playback (130 s), and recovery periods (120 s) (Fig. 1). The thermal recordings were conducted during baseline and recovery peri- od, but not during stimulus-playback periods (i.e. no giving of fruit pieces during this period). This is because we expected that chimpanzees would show overt responses to the stimuli during the playback (e.g. looking back, leaving the mesh) and that no reliable thermal re- cording was possible. After the stimulus-playback, the experimenter asked the chimpanzee to come closer to the mesh and established the post-measurement as soon as possible. This post-measurement occurred, on average, 14.7 s (±8.8 SD) after the stimulus offset, followed by the recovery period for 120 s. The nasal temperature was measured every 30 s in baseline (i.e. −30, 0 relative to the stimulus onset) and recovery periods (i.e. 0, 30, 60, 90, 120 s relative to the stimulus offset) using a region of interest (ROI) covering the nasal tip of chimpanzee. This was conducted offline and semi-automatically using the FLIR ResearchIR Software. The optimal frames for thermal coding, which are defined below, were searched within ±10 s (30% of time window) of each measurement time-point. When the optimal

frame was unable to be detected, null value was assigned for that time-point. In Experiment 1, only 3 measurement time-points (0.6% of all data) were coded as null values, and thus those points were later linearly interpolated after visually inspecting that no data distortion occurred due to the interpolation.

Thermal coding criteria. One challenge of our coding was to find the reliable temperature values in a relatively noisy condition, where chimpanzees could move their heads freely and a metallic (cold) mesh partly blocked the thermal images. To cope with this issue, we set the following criteria. (1) We used the minimal temperature in the nasal-tip area (i.e. in a region-of-interest, ROI) instead of the average temperature of a whole ROI area. This is to avoid the difficulty in finding the optimal size and position of ROI in each frame where the facial angle and position could vary. (2) When searching for the optimal frame for coding, we avoided the frames in which the chimpanzee was breathing in (this can be identified as a sudden decrease in temperature near nostrils) in order to avoid erroneously detecting the air temperature near nostrils. (3) After detecting the candidate optimal frame and the minimal value in the nasal tip, we checked if the detected value (and the spatial position where the minimal value was detected) does not abruptly change across temporally adjacent frames. In case that we detected such abrupt changes, we suspected the presence of artifact (e.g. due to breathing) and re-searched for the optimal frame. (4) In addition to these criteria, according to the recommendation by Ioannou et al. [22], we selected frames in which the face was in a constant angle throughout the whole session and avoided the frames in which the face was turned away from that angle (N 45°). (5) We also avoided the frames in which the facial image was blurred due to the head motion. With these criteria, we estimated the inter-coder reliability with an additional coder, who was uninformed about the experimental conditions, using 9 sessions of data (one session was randomly chosen from each chimpanzee, and each session had 5 measurement time-point; thus total 45 data-points). We found a high degree of agreement, as measured by the intraclass correlation coefficient (ICC = 0.99). See Fig. S2 for the example images which passed (or failed to pass) the criteria.

Behavioral measurement. Another experimenter recorded the chimpanzee's behavior using a video camera (Sony HDR-SX560). We measured the number of steps (the positional change of hind-limbs) as a proxy to the physical activity. We also measured the presence or absence of excitement behavior in each session (coded as 1 or 0). The excitement behavior was defined here as one of these behaviors; the facial expression (grin or pout face), vocalization (hooting), piloerection, body swaying, or charging to the walls. We estimated the inter-coder reliability with an additional coder uninformed about the experimental conditions for these two measurements using the data from 18 sessions (two sessions were randomly chosen from each chimpanzee). The intraclass correlation coefficient for the number of steps was 0.98, and the Cohen's Kappa for the presence of excitement behavior was 1.

Saliva collection and cortisol measurement. Salivary samples were collected three times in a session. The baseline sample was taken immediately after the chimpanzee came into the testing room (i.e. during the resting period). Post-stimulus salivary samples were taken at both 10 min and 20 min after the stimulus offset based on findings in humans that peak cortisol levels can be found 10–40 min after a stimulus [37]. The participant chimpanzees were given an oral swab (SCS, Salimetrics Europe Ltd., UK) and asked to chew on it several times and return it to the experimenter. Powdered sugar was sprinkled on the swab to motivate them to chew on it, and a fruit piece (grape) was given as a reward for the returning of the swab. The same amount of sugar and fruit re- ward was used for the three samples

collected in each session. After the saliva collection, the swabs were placed into a plastic container and stored at − 20 °C until analysis. To extract the saliva from the swabs, we centrifuged the tubes at 4700 rpm for 10 min and froze the samples at −20 °C. After thawing, samples were again centrifuged at 3000 rpm for 5 min and 30 μl of the supernatant were diluted 1:8 with assay buffer. Dilutions were run in duplicates with a Cortisol En- zyme Immunoassay provided by Coralie Munro (AK R4688 University of California-Davis, CA, USA). Serial dilutions of a pooled saliva sample and cortisol standards gave parallel displacement curves. Inter-assay coefficients of variation for high and low concentration controls were 5.6% and 9.4%. Intra-assay coefficients of variation for high and low concentration controls were 4.9% and 9.3%.

Data analysis. Raw thermal data were first visually inspected to check the overall time trend (see Fig. S3). In most of the sessions, we observed that the nasal temperature was stable across time in each session, and the temperature drop occurring in the middle of the session typically recovered to the baseline level at the end of the session. How- ever, in some of the sessions, we observed a linear increasing trend in the nasal temperature probably due to the fact that the initial nasal temperature was incompletely stabilized at the beginning of the session (note that we prepared the resting period for 10−15 min before the be- ginning of the session. However, incomplete stabilization occasionally occurred due to the very low nasal temperature of chimpanzees before the resting period or the unexpected locomotive activities by chimpanzees during the resting period). Such increasing trends in temperature could lead to a severe artifact in the estimation of temperature change in response to the stimuli. We thus removed all linear trends from the data using a linear-detrend function (see Fig. S4 for the detrended data). Then, the mean of pre-stimulus data (−30−0 s) was subtracted from the data collected after the stimulus offset (10−120 s) to correct for the baseline.

To examine the effect of condition (Cond) and stimulus repetition (Rep) on the temperature data at the post-measurement point (immediately after the stimulus offset), we used a general linear mixed model (GLMM, R-version 3.1.3 function "lmer" in a package "lmerTest", with binomial error structure and logit link function). No data transformation on the temperature data was conducted because they were near normally distributed. We included Cond and Rep as fixed effects and the subject (Subj) as a random effect. We also included the random slopes of Cond and Rep and its correlation with the random intercept. We kept them in the final model as they were significant (likelihood ratio test; $\chi^2 = 14.94$, $df = 6$, $p = 0.021$). We also included the interaction term between Cond and Rep into the model but removed it from the final model because they were not significant (likelihood ratio test; $\chi^2 = 4.28$, $df = 2$, $p = 0.12$); note that the presence of non-significant interaction makes the interpretation of main effects difficult [38,39]. In addition, to control for the effect of general activity on the nasal temperature, we included the number of steps (Step) into the model as a covariate. To distinguish within- from between-subject effects in Step (note that these are not distinguished by the random terms in the model), we used a technique called "within-subject centering" [40]; we included individual means and within-subject variations around the means as separate fixed effects. The significance of the full model as compared to the null model (excluding Cond from the final model but keeping all the random terms) was calculated using a likelihood ratio test (R function "anova" with argument test "Chisq"). We additionally obtained p-values for the individual effects from the "lmerTest" package (calculated based on Satterthwaite's approximation). We then examined the effect of Cond and Rep on Step in a separate GLMM (Subj as a random effect). The random slopes of Cond and Rep and the interaction between Cond and Rep were removed from the final model as they were not significant in this additional analysis. Finally, to examine the effect of Cond and Rep on the cortisol data (respectively at

10-min and 20-min measurement points), we conducted an additional GLMM (Subj as a random effect). The random slopes of Cond and Rep and the interaction between Cond and Rep were removed from the final model as they were not significant also in this analysis.

For all the models mentioned above, we visually inspected the distribution of residuals and confirmed no severe violation in the assumption of normally distributed and homogeneous residuals. By inspecting Variance Inflation Factors (R function "vif" in a package "car"), we also confirmed that collinearity was not an issue. Finally, to check the model stability, we excluded the subjects one-by-one from the data and inspected the estimates and fitted values in each manipulation and confirmed that there was no particular influential subject in the data.

Result

Thermal measurement

Overall, the full (final) model was significantly distinguished from the null model (likelihood ratio test; $\chi^2 = 6.07$, df = 2, p = 0.047); we thus confirmed the significant effect of condition. The nasal temperature dropped about 1−1.5 °C in the chimp-fighting condition, and about 0.5 °C in the other two conditions (Figs. 2; 1a). The effect of stim- ulus repetition was not significant (estimate ± SE = 0.057 ± 0.25, p = 0.0037). Both within- and between-subject effects of the number of steps were significant, although the between-subject effect (estimate ± SE = −0.010 ± 0.004, p = 0.013) was smaller than the within-subject effect (estimate ± SE = −0.041 ± 0.011, p = 0.0037). As the chimpanzees walked more, their nasal temperatures dropped more strongly (Pearson's r = −0.42, n = 54, p = 0.0016; Fig. 2b). These decreases in nasal temperature recovered to the baseline level at about 2-min after the post-measurement point (Fig. 2c). According to the 95% confidence interval at each measurement point (Fig. 2b), the effect remained up to 90 s (but most reliably up to 30 s) after the first measurement point (about 15 s after the stimulus offset) in the chimp-fighting condition.

Behavioral measurement

Chimpanzees showed excitement behaviors differentially between conditions (Table 1; Fisher's exact test, p = 0.010); their excitement behaviors were more frequently observed in the chimp-fighting than orangutan long-call condition, and no excitement behavior was observed in the no-sound condition. In GLMM with the number of step as a response variable, the full model was marginally significant as com- pared to the null model (likelihood ratio test: $\chi^2 = 5.27$, df = 2, p = 0.072). Thus, the number of step tended to differ between conditions (mean ± SD, chimp-fighting 55.5 ± 27.0, no-sound 36.0 ± 29.8, long- call 47.7 ± 37.1).

Salivary cortisol measurement

The salivary cortisol did not particularly respond to the playback stimuli in this experiment (Fig. 3). In GLMM, the full model was not significantly distinguished from the null model either in 10-min (likelihood ratio test: $\chi^2 = 0.50$, df = 2, p = 0.78) or 20-min measurement point ($\chi^2 = 1.03$, df = 2, p = 0.60).

Discussion

Chimpanzees showed the decrease in nasal temperature particularly in response to the conspecific vocalizations during fighting (chimp- fighting condition). They showed the decrease in nasal temperature in the other two conditions, orangutan long-call and no-sound condition, but to a lesser degree than in the chimp-fighting condition. To some ex- tent, the decrease in nasal temperature was caused by the physical activities that chimpanzees made during the stimulus presentation, as the amount of locomotive activities was negatively correlated with the temperature change. However, the physical activities alone cannot ex- plain the differences in nasal temperature between conditions partly because they were co-varied out in a mixed model, and also because the changes in physical activity were only marginally related to the presented stimuli or conditions. The decreased temperatures recovered to the baseline in about 2 min.

Chimpanzees showed excitement behaviors most frequently in the chimp-fighting condition, less frequently in the orangutan long-call condition, and least frequently (none) in no-sound condition. These results thus support the idea that excitement (the activation of sympathetic nervous system, SNS) caused both behavioral and physiological changes in chimpanzees. However, we did not observe any changes in salivary cortisol level in chimpanzees. The most likely explanation for this difference is that the playback stimuli that caused the SNS activity did not lead to the activation of the hypothalamus–pituitary–adrenal (HPA) axis. In support with this idea, several studies with human participants showed that the SNS activity, as measured in salivary alpha- amylase level, was not necessarily accompanied with the HPA activity, as measured in salivary cortisol level [42-45].

There are at least three remaining questions in Experiment 1. (1) It remains unclear whether the decrease in nasal temperature occurs even without any physical activities. (2) It remains unclear how quickly the nasal temperature dropped after the stimulus onset. (3) If the de- crease in nasal temperature was driven by the activation of SNS, we should observe the responses in the other SNS measures such as galvanic skin conductance [3] and heart-rate/heart-rate variability.

Experiment 2

To answer the remaining questions raised above, we conducted an additional experiment in another research facility, Kumamoto Sanctuary, Japan. In this facility, a familiar experimenter can be in the same experimental booth with the chimpanzees, and therefore can control the chimpanzees to some extent on verbal commands and rewards (with- out physically restraining their bodies). With this unique set-up, Experiment 2 aimed to replicate and extend the results from Experiment 1. In each condition, three chimpanzees watched the videos of conspecific fighting (with screaming and barking vocalizations), conspecific resting (with natural background noises), or blank screen (no sound). The presentation of naturalistic videos is another common research tool in primate studies [e.g. [21,46]]. Chimpanzees sat on a small stool so that we could avoid their locomotive activities. This also enabled us to keep tracking the changes in nasal temperature before, during, and after the stimulus presentation.

Additionally, as one of the three chimpanzees was already trained to accept the electrodes (sponges, tapes, and cables) on her body (i.e. not to take them away during a session, without being physically re- strained), we collected electrocardiography (ECG) data from this chimpanzee simultaneously with

the thermal recording. Several previous studies have examined the heart-rate (HR) response to social stimuli and reported a rather complex pattern of HR changes in chimpanzees. As our stimuli (conspecific fighting including screams and barks) are potentially both threatening and engaging, we could expect both in- crease and decrease in HR. An alternative, more recently developed method is the frequency analysis of heart-rate variability (HRV). HRV is the naturally occurring variation of beat-to-beat intervals (i.e. the intervals between R peaks, or RR intervals, in ECG recordings) that occurs during a breathing cycle (i.e. respiratory sinus arrhythmia) [47]. This naturally occurring variation of beat-to-beat intervals is a reliable measure of parasympathetic nervous activities, and can be measured as the relative power of high-frequency (HF) component in a HRV spectrum [16,17]. Thus, during excitement, the HF component in a HRV spectrum typically decreases in participants. In this experiment, we thus expected the decrease in HF power during the presentation of fighting videos in the chimpanzee.

Method

Participant

Three chimpanzees (females; age 6, 9, 18 years of age) participated in this study (Table S1). They were naïve to the procedures of thermo- imaging. They lived in Kumamoto Sanctuary (KS), Japan. On each day of testing, each chimpanzee was called into a separate testing room, where various cognitive tests were conducted on a routine basis. These chimpanzees had an established relationship with one of the experimenters from youth and were able to stay with the experimenter in the same experimental booth. One of the chimpanzees was previously trained to accept electrodes attached to her body [48]. Thus, we con- ducted an ECG recording with her simultaneously with the thermal recording.

Ethics statement

Their living areas were large and complex enough for them to rest, exercise, and socialize with the other group mates [49]. They received fresh fruits, vegetables, nuts and leaves distributed in three main meals and occasional enrichment programs [as described in Matsuzawa et al. [50]]. Water was available ad libitum throughout the day. The chimpanzees voluntarily participated in this study and were never food or water deprived. Animal husbandry and research complied with the international standards and the local guidelines which are strictly adhered to the national laws of Japan. See Supplemental material for these details.

Stimuli

We presented to chimpanzees two sets of movie stimuli in Experiment 2 (total six movie files). We avoided repeating the same stimuli in this experiment in order to avoid habituation to the video stimuli [46]. The first sets consisted of three 180-s movies in three conditions; one movie in each condition. In the chimp-fighting condition, the movie depicted a severe "grab and bite" fight of chimpanzees (unfamiliar individuals from WKPRC, Germany). The sound included the screams and waa-barks. The previous studies have confirmed that chimpanzees show excitement behaviors and great interests to the videos of conspecific fighting [by unfamiliar individuals; [46]]. In the chimp-resting condition, the movie depicted rested chimpanzees (unfamiliar individuals from WKPRC) grooming with each other. The sound included the natural background noises consisting of murmuring of water streams and bird squeaks. In the blank-screen condition, the movie was a black image with no sound. The second sets of stimuli were the same as the first sets except that the movies depicted the different events from the first sets in the chimp-fighting and chimp-resting conditions. The images and

sounds were respectively played in a monitor (Acer, 23-inch, 53 × 30 cm, 1280 × 720 pixels) and a speaker (the same as Experiment 1).

Procedure

Thermal measurement. We used the same thermo-camera (FLIR T650sc) to record the nasal temperature of chimpanzees in Experiment 2. One of the experimenters brought the camera, monitor, and speaker with connecting cables into a small experimental booth, which was located in the testing room and was surrounded by transparent polycarbonate panels for the purpose of observation from outside. Another experimenter stayed outside of the booth and controlled the apparatus- es using a laptop. The camera was placed on a tripod at about 1.5 m from the chimpanzee. The chimpanzees sat on a small stool, and the experimenter gave to the two chimpanzees (Natsuki, Mizuki) small pieces of time-consuming foods (in-shell peanuts, in-shell sunflower seeds, and blocks of sugarcanes) as rewards for not leaving from the stool. Consumption of these foods did not change the nasal temperature of chimpanzees (but see Supplementary Method for the results from pre- liminary sessions in which consumption of large amount of foods changed their nasal temperature). The other chimpanzee, Hatsuka, did not receive any rewards (because she was able to stay on the stool during the session without the rewards and also because the same amount of foods did decrease her nasal temperature unlike the other two chimpanzees, presumably due to her smaller body size).

Each testing session lasted for about 15−20 min. Only one session (the presentation of a stimulus) was conducted on each day for each participant. All sessions were conducted in 12−13 pm, November−December 2014, and March−May 2015. The coldest term (January−March) was avoided because the air temperature consider- ably lowered the baseline nasal temperatures of chimpanzees.

The testing room was kept in a constant temperature and humidity throughout each session with no direct sunlight or ventilation. The temperature and humidity varied 18−22 °C and 50−60%, respectively, across the testing days. As in Experiment 1, in most of the testing days, the initial temperature of nasal tip was lower than that of other facial areas in the chimpanzees, probably due to the excitement and physical activities when they were called into the testing room (and also due to the cold environmental temperature in their living areas). Therefore, as in Experiment 1, we prepared a 10−15 min resting period before each daily session until the chimpanzees showed no behavioral sign of excitement or anxiety (and until their nasal temperatures were stabilized as much as possible).

The daily session was divided into three phases, baseline (30 s), stimulus-playback (180 s), and recovery periods (120 s). The thermal recordings were conducted throughout a whole session. The nasal temperature was measured every 10 s (i.e. 33 data points in −30−300 s period) using the same method and criteria as Experiment 1. The optimal frames for coding were searched within ±3 s (30% of time window) of each measurement time-point. We were unable to code only four measurement points (0.6% of all data); these measurement points were later linearly interpolated after visually inspecting that no data distortion occurred due to the interpolation. We estimated the inter-coder reliability with an additional coder uninformed about the experimental conditions using the data from 3 sessions (one session, with 33 measurement time- points, was randomly chosen from each chimpanzee; total 99 data- points) and confirmed that the intraclass correlation coefficient was reliably high (0.95).

Behavioral measurement. Two video cameras (Sony HDR-SX560) recorded the whole areas of experimental booth during the sessions. As in Experiment 1, we recorded the chimpanzees' overt

excitement behavior. Chimpanzees did not show any locomotive movements in Experiment 2. See Movie 2 for examples of overt behaviors.

Heart-rate (HR) measurement. An electrocardiograph (PowerLab, ADInstruments Japan Inc.) recorded the heart-beat signals from the chimpanzee, Mizuki. Three electrodes (Ambu Blue Senser P) were attached to both wrists (positive and negative) and one of the feet (earth) of the chimpanzee. A laptop (HP ENVY17) with a LabChart soft- ware (version 7.3.1, ADInstruments) and an ECG (electrocardiography) module recorded the ECG signals and automatically detected R peaks. The software then automatically detected potential artifacts and re- moved them from the data (b 5% of data loss in each session).

Data analysis. We first visually inspected the raw thermal data to check the overall time trend (see Fig. S5). We found linear trends in some of the sessions and thus removed them from the data (see Fig. S6) to avoid the severe artifacts due to the temperature recovery, as in Experiment 1. The data were then baseline corrected. To examine the effect of condition and stimulus (i.e. two stimuli presented respectively at first and second sessions) on the temperature change at the post-measurement point (10 s after the stimulus offset), we used a GLMM with condition and session as fixed factors and subject as a random factor, as in Experiment 1. The random slopes of Cond and Rep and the interaction between Cond and Rep were removed from the final model as they were not significant. We also checked the model assumptions as in Experiment 1 and confirmed no severe violation.

Heart rate (HR) was calculated from the RR-intervals and averaged for each 10-s time-window (33 data-points in −30–300 s period) and then baseline corrected. Heart-rate variability (HRV) was calculated for each 1-min time window (6 data points in − 60–300 s period) using the frequency-domain method in a software [Kubios HRV; [51]], and then baseline (− 60–0 s) corrected. In the software, a spectrum for the RR-interval series was estimated using Welch's periodogram and divided into very low frequency (VLF: 0–0.04 Hz), low frequency (LF: 0.04–0.15 Hz), and high frequency (HF: 0.15–0.4) bands, which yielded the relative power (%) of each band as outputs. HF band is known to reflect the parasympathetic nervous activity (due to the respiratory sinus arrhythmia) [47].

Result

Thermal measurement

Overall, the full model was significantly distinguished from the null model (likelihood ratio test: $\chi^2 = 12.52$, df = 2, p = 0.0019); we thus confirmed the significant effect of condition. Consistent with Experiment 1, the nasal temperature dropped about 1–1.5 °C in the chimp- fighting condition, and about 0–0.5 °C in the other two conditions at the post-measurement point (10 s after the stimulus offset; Fig. 4). The effect of stimulus was also significant (estimate ± SE = −0.87 ± 0.26, p = 0.0052). The nasal temperature dropped more intensely in response to the second as compared to the first stimulus (Fig. 4a). According to the 95% confidence interval at each measurement point (Fig. 4b), there was a detectable change (from the baseline) 30 s after the stimulus onset, and the effect remained up to 80 s (but most reliably up to 40 s) after the stimulus offset in the chimp-fighting condition.

Behavioral measurement

Chimpanzees showed excitement behaviors (hitting the monitor, swaying body, pout face), in 4 out of 6 sessions (2 for Natsuki, 2 for Hatsuka) in the chimp-fighting condition, 2 out of 6 sessions (Natsuki) in the chimp-resting condition, and 0 out of 6 sessions in the blank- screen condition (no statistical test was conducted due to the lack of sufficient samples). Notable physical activities were observed in 3 cases, by the same chimpanzee, Natsuki, in the chimp-fighting condition; she hit the monitor by swinging her arm several times; 60/140 and 130 s after the stimulus onset, respectively in the first and second sessions. No chimpanzee showed locomotive activities.

HR measurement

Fig. 5 shows the chimpanzee's (Mizuki) HR and HRV changes. The mean HR did not particularly differ between conditions or stimulus sets. The relative power of HF band of a HRV spectrum was lower in the chimp-fighting condition than in the other two conditions consistently across time in the first stimulus set (Kruskal–Wallis test: $\chi^2 = 10.82$, df = 2, p = 0.0089) and the second stimulus set ($\chi^2 = 9.68$, df = 2, p = 0.015) (p values were Bonferroni corrected).

Discussion

Chimpanzees showed the decrease in nasal temperature in the chimp-fighting condition and to a lesser degree in the chimp-resting condition. No change occurred in the no-sound condition. The second sessions yielded stronger responses of chimpanzees than the first sessions. This difference is presumably due to the relatively low baseline nasal temperature in the first sessions (i.e. the influence of cold climate when the first session was conducted; see Fig. S5). It may be also related to the difference in the stimulus content, as chimpanzees exhibit differential responses to a conspecific scream depending on its severity [52]. Averaging over the two sessions, the nasal temperature dropped almost linearly up to 1.5 °C in 2 min (in 3-min videos), and recovered to the baseline level in 2 min. Therefore, overall, we replicated the results from Experiment 1.

Chimpanzees did not show locomotive activities in Experiment 2 (and the nasal temperature did not change in the control blank-screen condition) unlike Experiment 1. Only in three cases, one chimpanzee showed overt limb movements (hitting the monitor) in the chimp- fighting condition, but importantly, this occurred only after her nasal temperature dropped to some extent (i.e. after a minute). Two of the three chimpanzees did not show the overt limb movements but nonetheless showed the decrease in nasal temperature in the chimp- fighting condition.

The mean heart-rate (HR) did not differ between conditions or stim- ulus sets. However, the chimpanzee changed her HR rather abruptly, sudden increase and decrease, in the chimp-fighting condition. This is presumably due to the conflict between the HR increase caused by her excitement and the HR decrease caused by her attention to the stimuli. The high-frequency power of a HRV spectrum was consistently lower in the chimp-fighting than chimp-resting/blank-screen condition. These results thus indicate the decreased activity of parasympathetic nervous system in the chimp-fighting condition in the chimpanzee.

General discussion

We established thermo-imaging with chimpanzees at two of the re- search facilities differing in their experimental set-ups. We then characterized the psychological, physical, and environmental factors that affected their nasal temperature. In the WKPRC facility, the chimpanzees freely moved during the stimulus presentation. We thus conducted thermal measurements by attracting them to the mesh with pieces of food before and after the stimulus presentation. Chimpanzees showed the decrease in nasal temperature particularly in response to playbacks of fighting vocalizations of groupmates. The locomotive activities that chimpanzees made during the stimulus presentation also affected the nasal temperature, although this effect alone could not explain the chimpanzees' differential responses to stimuli as indicated by a mixed model including the number of steps as a covariate. Excitement behaviors were most frequently observed during playbacks of fighting vocalizations. Salivary cortisol changes were not detected at least within 20- min after the stimulus offset.

In the KS facility, the chimpanzees sat on a stool during the stimulus presentation. We thus conducted thermal measurements before, during, and after the stimulus presentation. Chimpanzees showed the decrease in nasal temperature particularly in response to the videos of conspecific fights. The nasal temperature dropped without or before any physical activities. The high-frequency power of a HRV spectrum decreased in response to the videos of conspecific fights, suggesting the inhibition of parasympathetic nervous system (or the activation of sympathetic nervous system).

In this study, we identified three major factors that affected the nasal temperature of chimpanzees; physical or psychological stimulation, homeostasis that recovers the lowered temperature, and cold environmental temperature. As we were interested only in psychological factors, the other factors needed to be controlled out. The potentially confounding factors and the corresponding controlling methods were summarized as follows. (1) Locomotive activities lowered the nasal temperature of chimpanzees. They were co-varied out post-hoc in a mixed model in Experiment 1, and were minimized in a set-up of Experiment 2. (2) Quick consumption of large amount of foods (e.g. a banana) lowered the nasal temperature of chimpanzees presumably due to the meal-induced activation of sympathetic nervous system [34,36]. Thus, several pilot tests were conducted to find the amount of foods that did not seem to trigger the sympathetic nervous activity in Experiment 2 (see Supplemental Method for the details). (3) Cold environmental temperature (in winter time) affected the baseline nasal temperature in chimpanzees. As a consequence, the nasal temperature tended to rise linearly throughout a test session (independently of the stimuli) to reach a thermal equilibrium with the experimental room. To avoid this, pre-session resting periods were prepared as long as possible (longer than 10−15 min). However, when we observed such linear trends nonetheless in the analysis, we detrended the raw data post- hoc to better capture stimulus-related temperature changes (Experiments 1 and 2).

Despite the fact that these confounding factors partly limit the use of thermo-imaging as a physiological measure of emotion, we found that thermo-imaging was nonetheless most useful among several measures that we tried in this study; observation of expressive behaviors, measurement of salivary cortisol, and electrocardiography (ECG) recordings. (1) In Experiment 1, the frequency and pattern of behaviors indicated the chimpanzees' excitement during a stimulus presentation. However, there were large individual differences in the form of expressions across individuals. For example, in Experiment 1, some individuals showed vigorous activities including the vocalization and charge into the walls, but the others were immobile and silent, and showed only facial expressions (e.g. grin, pout). In Experiment

2, two of the 3 individuals showed no obvious activities. Yet, they nonetheless showed the de- crease in nasal temperature (and the change in heart-rate variability, HRV). (2) In Experiment 1, salivary cortisol change was not detected. Thus, although our stimuli seemed to trigger the activation of sympathetic nervous system, they did not seem to trigger that of hypothalamus–pituitary–adrenal (HPA) axis. (3) In Experiment 2, one chimpanzee participant was found suitable for ECG recording, and her HRV changes indicated her excitement during a stimulus presentation. However, the other chimpanzees rejected the electrodes, and thus we were not able to collect ECG data from them.

HR/HRV as well as galvanic-skin-conductance response [3] were two of the most common methods in the studies of emotion with humans, due to their sensitivity and short response time window. However, these features are rather shortcomings in experimental set-ups adapted for great apes because, typically in these measures, even small physical activities of subjects (e.g. standing up) cause severe noises, and the response delay is minimal. In contrast, the change in skin temperature is essentially an accumulative phenomenon (the addition of effect each time). This leaves us a possibility of controlling physical activities post-hoc (by co-varying the physical activities in a model, or by comparing between experimental and control conditions). In addition, as the temperature changes last at least over several ten seconds before it re- covers into the baseline level in 2 min, we have enough time to establish the post-measurement points.

Thermo-imaging should be useful in the future studies because it can objectively measure the internal psychological state of individuals. There are several topics that may be suitable for thermo-imaging. First, in primates, the degree of excitement/stress experienced by an in- dividual reflects the individuals' understanding about the other individuals' mental state and social relationships. For example, chimpanzees showed more behavioral signs of distress when they found an intention of teasing in the experimenter [53]. Chimpanzees selectively looked at the playback of conspecific screams based on the severity of fights in which the screams were recorded [52]. Thermo-imaging can be a useful physiological measure in addition to the existing behavioral measures. Second, in primates, the degree of stress experienced by an individual predicts how individuals and species make decisions and solve problems in a foraging context. Chimpanzees inhibit their responses to obtain an immediate reward in order to obtain a delayed better reward [54-56]. Bonobos differ from chimpanzees in this respect as they make more inpatient choices than chimpanzees [56]. Chimpanzees use com- plex tool-using more often than bonobos in the wild presumably due to their increased motivation (rather than their superior abilities) to manipulate objects [57]. Thermo-imaging may capture how individuals cope with the stress associated with the foraging task. Third, in primates, emotion has a regulatory function in social interaction. Primates keep an appropriate level of social distance (and eye contact) with the other individuals depending on their social relationships [58,59]. They reconcile each other after a severe fight to restore their relationships [60-64]. Implicit copying of another individual's emotion may lead to a better understanding of another's motivation and intention [65,66]. Sharing the same stress thorough emotional contagion may lead to con- solation or prosocial behavior [61,62,67-69]. Thermo-imaging could capture how the individuals behaviorally react to social stress in a com- plex social interaction. Forth, in humans, sexual arousal causes the in- crease in nasal and perioral temperatures [23,31,32], an opposite effect that we observed in this study. Sexual interest is one of the major behavioral drives in primates; yet its physiological and psycho- logical underpinnings remain relatively unclear [70]. An objective and handy tool to measure sexual arousal should open a new possibility to examine this relatively understudied emotion in nonhuman primates.

In conclusion, thermo-imaging can capture the psychological state of chimpanzees. There are several advantages in using this technique in future studies. (1) Contact-free method allows us to track the psycho- physiological changes without the necessity of electrodes. (2) A relatively slow response time window (2 min) allows us to have more time to establish the post-measurement. (3) The accumulative nature of effects allows us to control the effects of physical activities post-hoc. (4) The increase and decrease in facial temperature respectively inform us of specific psychological states of participants. Yet, as in the other physiological changes related to the autonomic nervous activities (e.g. heart-rate, galvanic response), the physical activities and environmental conditions have non-negligible effects on the temperature changes, and so we need to carefully control those co-factors to elucidate the in- ternal states of animals.

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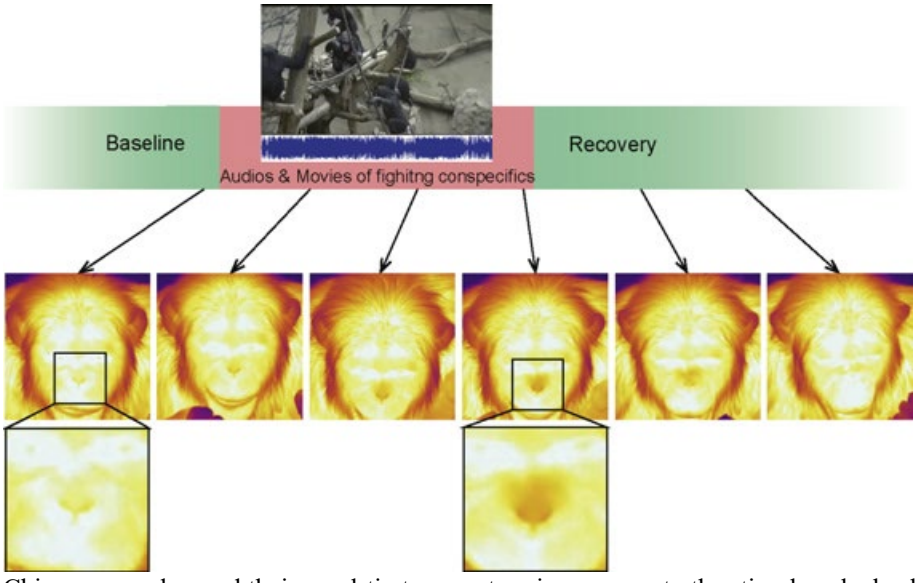
Graphic Abstract



Chimpanzees dropped their nasal-tip temperature in response to the stimulus playback.

Fig. 1. Example of chimpanzee thermal images and the decrease in nasal temperature in Experiment 1 (A) and Experiment 2 (B).

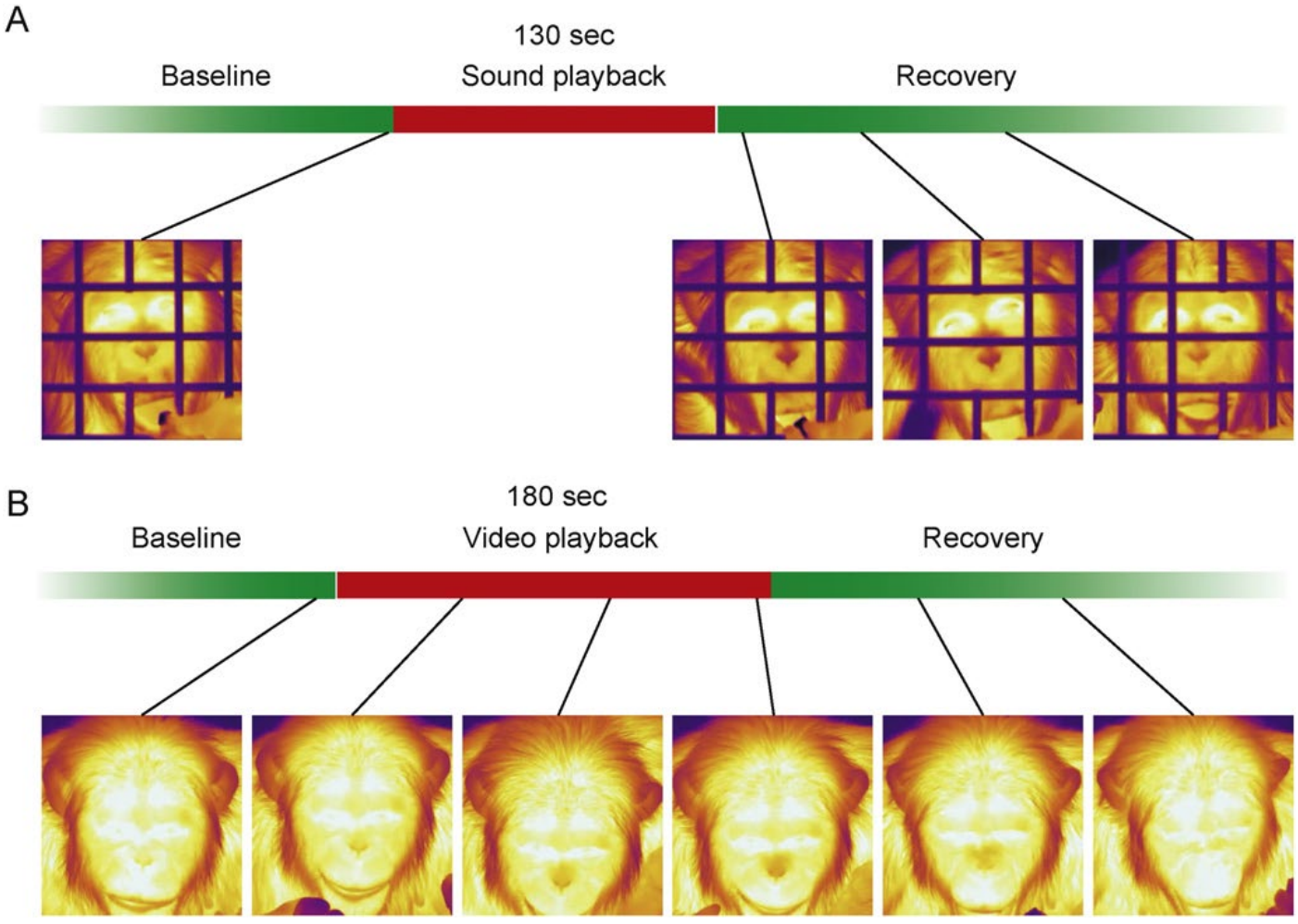


Fig. 2. The change in nasal temperature (from the baseline) at the post-measurement point in each condition (A). The correlation between the change in nasal temperature at the post- measurement point and the number of walk-steps observed during the stimulus presentation (B). The recovery of nasal temperature after the post-measurement point (C). Error bars denote ±95% confidence intervals, corrected for within-subject design [41].

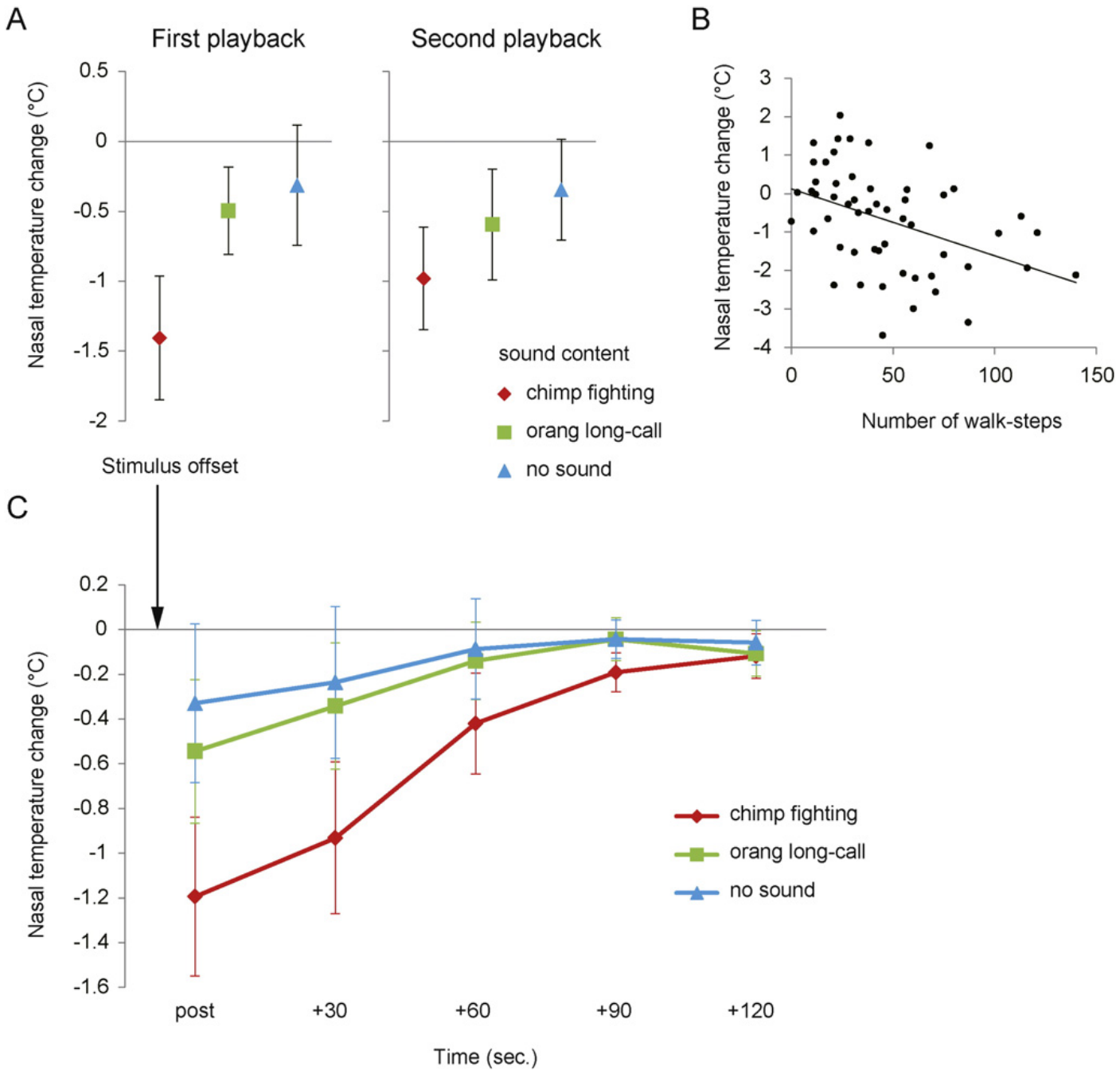

A
First playback
Second playback
Nasal temperature change (°C)
0.5
0
-0.5
-1
-1.5
-2
sound content
chimp fighting
orang long-call
no sound
B
Nasal temperature change (°C)
3
2
1
0
-1
-2
-3
-4
0
50
100
150
Number of walk-steps
C
Stimulus offset
Nasal temperature change (°C)
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
-1.2
-1.4
-1.6
chimp fighting
orang long-call
no sound
post
+30
+60
+90
+120
Time (sec.)

Fig. 3. The change in salivary cortisol (from the baseline) 10 min and 20 min after the stimulus offset. Error bars denote ±95% confidence intervals (corrected for within-subject design).

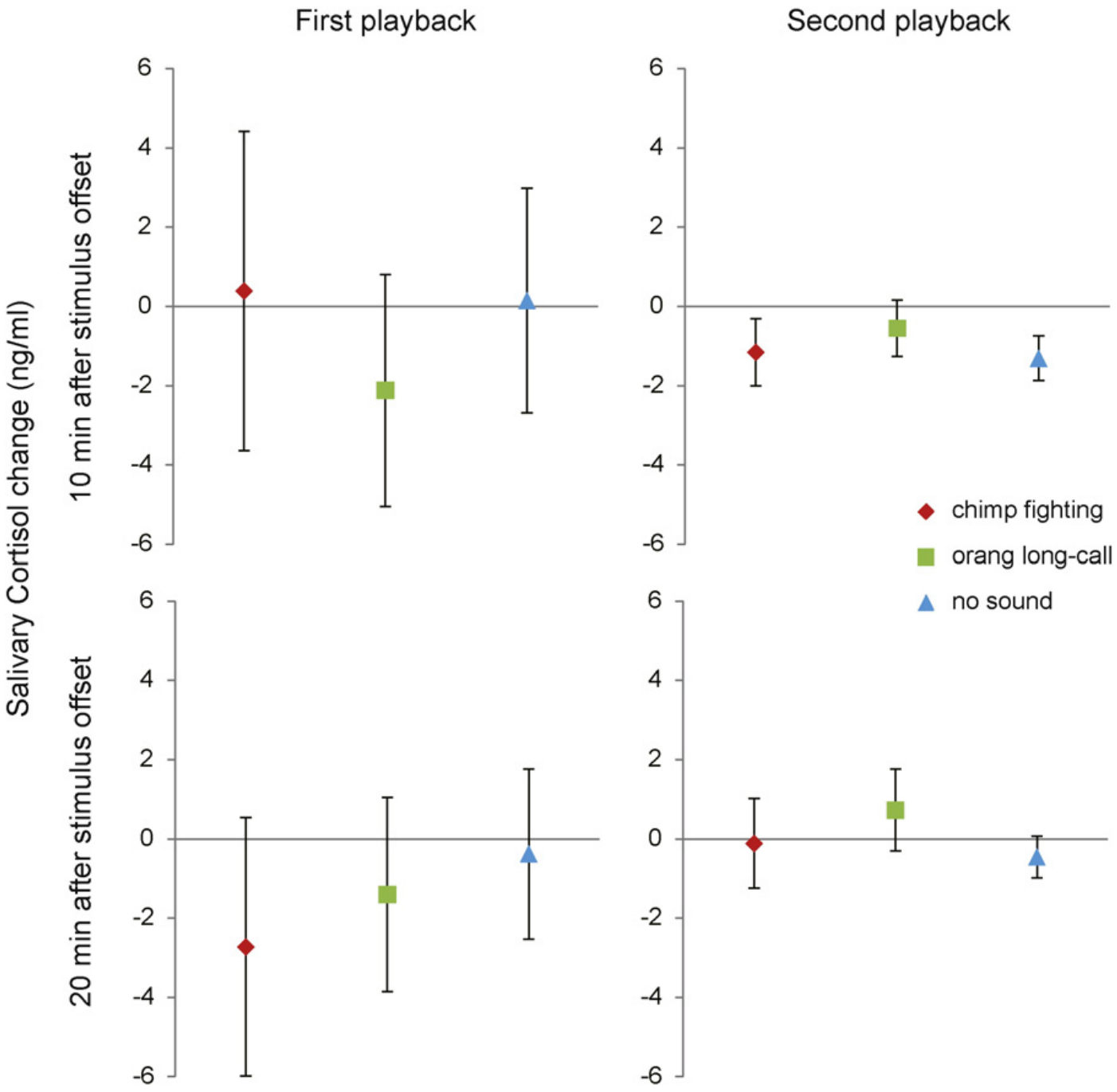


Fig. 4. The change in nasal temperature (from the baseline) at the post-measurement point (10 s after the stimulus offset) in each condition (A). The change in nasal temperature after the stimulus onset (B). Error bars denote ±95% confidence intervals (corrected for within-subject design).

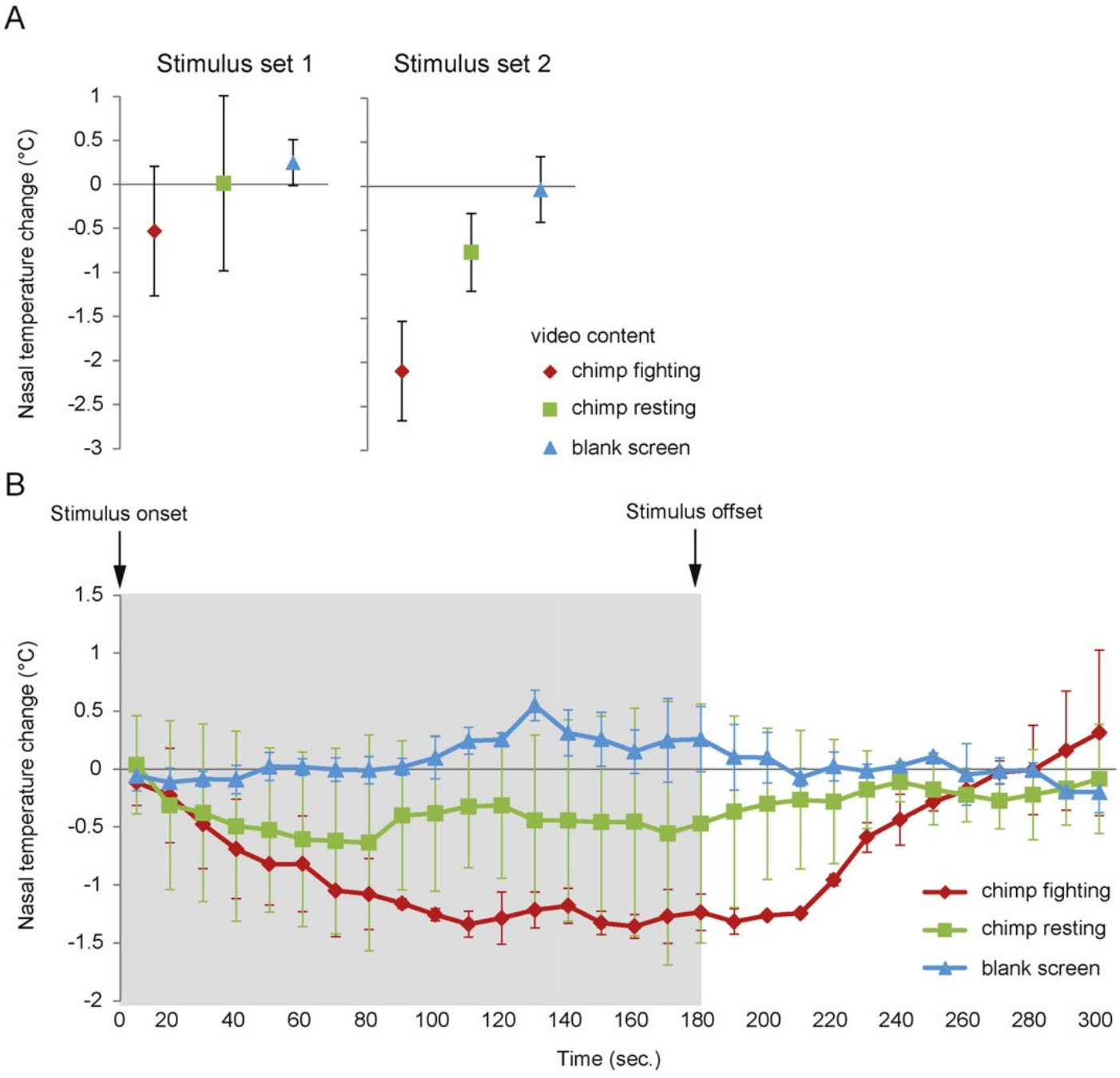


Fig. 5. The change in HR-mean (beat per minute, for each 10-s time-window) and HRV (% of high-frequency band) from the baseline. Also shown is the example of ECG signals, RR-intervals, and spectra.

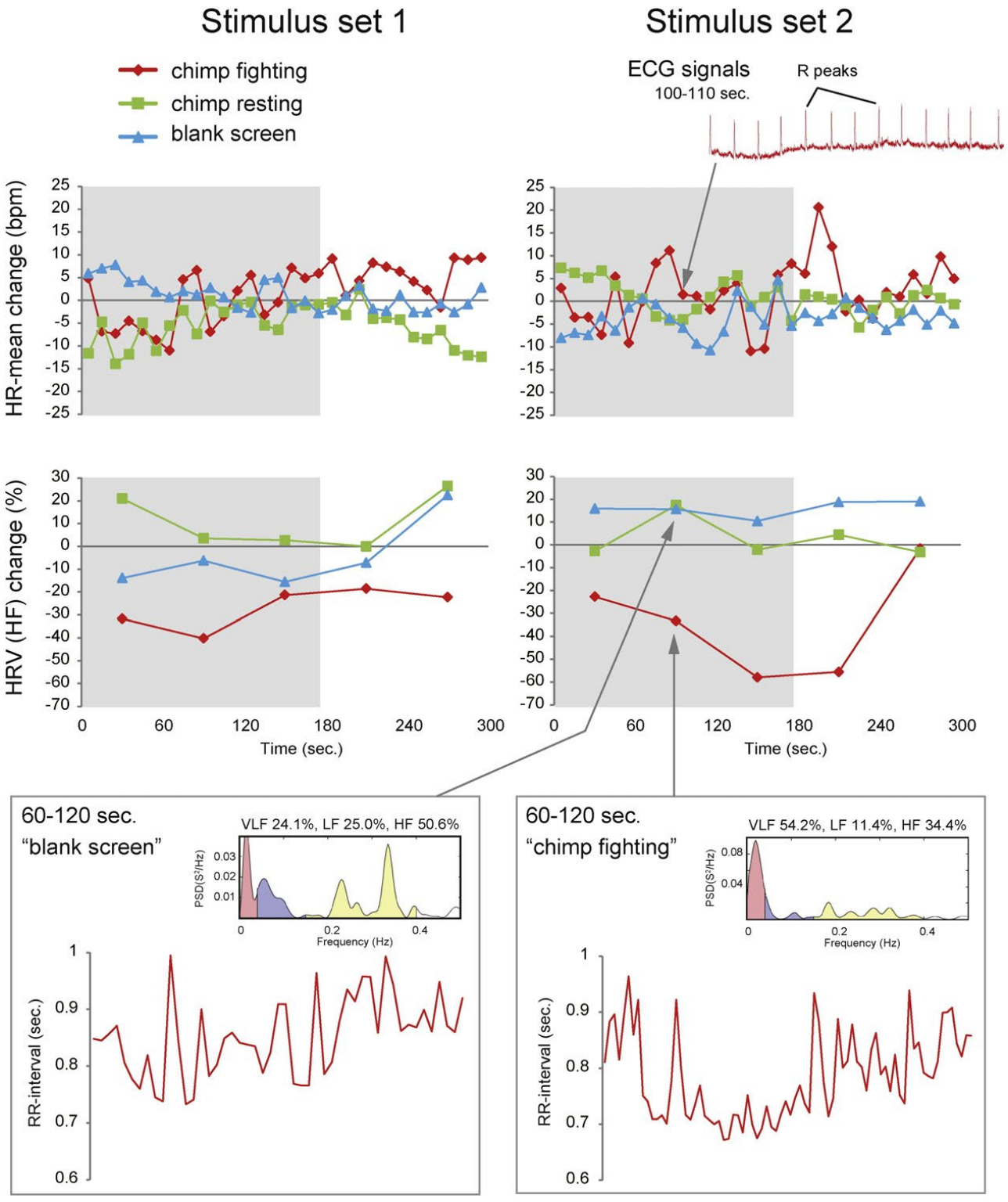


Table 1

Number of sessions in which excitement behaviors were observed in Experiment 1.

Condition	Observed	Total session
Chimp fighting	10	18
Orangutan long-call	4	18
No sound (control)	0	18